Roquefortine in the stomach contents of dogs suspected of strychnine poisoning in Alberta

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Abstract

From April to September 1990, submissions in Alberta veterinary diagnostic laboratories for which strychnine analysis was requested were tested retrospectively for roquefortine, a diketopiperazine alkaloidal tremorgenic mycotoxin. Roquefortine was found only in strychnine-negative samples. The specific origins of the fungal-contaminated specimens could not be determined. Of the six roquefortine-positive cases, four dogs that vomited prior to treatment recovered. Two dogs which died had significant amounts of stomach contents present at the time of postmortem.

At the present time only one veterinary laboratory in Canada is routinely testing for roquefortine when samples are negative for strychnine. It appears that a low diagnostic rate for this type of poisoning may be occurring due to incomplete testing. The inability to differentiate between roquefortine and strychnine poisoning on a clinical basis in five of our six cases emphasizes that an accurate causative diagnosis requires laboratory examination.

Research in rats and sheep has shown that the tremorgenic mycotoxins penitrem A and roquefortine are excreted through bile. Although further research is required, the submission of bile and intestinal contents is recommended if stomach contents or vomitus are not available for laboratory testing. Both of these mycotoxins should be tested for when strychnine analysis is negative as fungi may produce both toxins at the same time. In this study we were unsure if roquefortine alone or in combination with other toxins was responsible for our findings.

Résumé

Roquefortine décelée dans le contenu gastrique de chiens soupçonnés d'empoisonnement à la strychnine, en Alberta

Des échantillons soumis au laboratoire vétérinaire de diagnostic de l'Alberta au cours des mois d'avril à septembre 1990 ont été analysés rétrospectivement pour déceler la présence de roquefortine, une mycotoxine trémorgénique alkaloïde dikétopipérazine. La roquefortine a été trouvée seulement dans les échantillons exempts de strychnine. La source de contamination fongique des prélèvements n'a pu être identifiée. Des six chiens ayant présenté un résultats positif pour la présence de roquefortine, quatre ont vomi avant tout traitement et ont survécu alors que deux autres sont morts. À la nécropsie de ces derniers, on a pu observer un plein contenu gastrique.

Présentement, un seul laboratoire vétérinaire de diagnostic au Canada effectue de routine l'épreuve pour déceler la roquefortine lorsque les échantillons ont un résultat négatif pour la présence de la strychnine. Il est probable que le faible taux de diagnostic dans ce genre d'empoisonnement soit dû à une analyse incomplète. L'incapacité à différencier cliniquement l'empoisonnement à la strychnine de celui de la roquefortine dans cinq des six cas présentés démontre l'importance de l'analyse de laboratoire pour poser un diagnostic causal précis.

Les recherches effectuées chez le rat et le mouton ont démontré que les mycotoxines trémorgéniques penitrim A et roquefortine sont excrétées dans la bile. Même si d'autres études seraient nécessaires, il est recommandé de soumettre pour analyse des prélèvements des contenus intestinal et biliaire lorsque des échantillons de vomissures ou du contenu gastrique ne sont pas disponibles. Les deux mycotoxines mentionnées devraient être recherchées lors de résultats négatifs pour l'analyse de la strychnine puisqu'un fongi peut produire les deux toxines en même temps. Dans cette étude, les auteurs se demandent cependant s'il est possible de la roquefortine seule ou combinée à d'autres toxines soit responsable de leurs constatations.

(Traduit par Dr Thérèse Lanthier)

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Introduction

The purpose of this paper is to draw the attention of clinicians and pathologists to the possibility that tremorgenic mycotoxins are a source of poisoning to be considered as a differential diagnosis when dealing with suspected cases of strychnine poisoning. Tremorgenic mycotoxins are a diverse group of secondary metabolites produced by several fungal species that are found on decomposing organic material (1). The toxins cause muscle tremors and, depending on the animal species, toxin type, and concentration ingested, may result in ataxia, tonic convulsions, and death. Examinations for the presence of the two most commonly reported tremorgenic mycotoxins in dogs, namely penitrem A and roquefortine, should be undertaken when analysis for strychnine is negative. Negative results for tremorgenic mycotoxins would also indicate that examination may be advisable for metaldehyde, bromethalin-rodenticide, organophosphates, carbamates, chlorinated hydrocarbons, zinc phosphide, methylated xanthines (such as caffeine, theophylline, and theobromine), pyrethroids, and the insecticide diethyltoluamide.

In Alberta, the most common cause of poisoning in dogs is strychnine (2). In some cases, this finding implies malicious intent (3). Since similar clinical signs

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are common to both strychnine and tremorgenic mycotoxins, it is imperative that a correct diagnosis be made by laboratory analysis. Although our toxicology laboratory has previously identified roquefortine in the stomach contents of dead dogs, we have been unsure of its significance (4). In view of the fact that many histories indicating suspected strychnine poisoning are accompanied by stomach contents that test negative for this alkaloid, we concluded that some unidentified compounds were inducing strychnine-like clinical signs.

The first case of roquefortine poisoning linking clinical signs with this tremorgenic mycotoxin in a dog was reported in 1988 (5). Previous reports had indicated that penitrim A was the most common mycotoxin inducing clinical signs similar to those of strychnine poisoning in dogs (6). To determine the significance of finding roquefortine in the stomach contents of dogs, all submissions suspected to contain strychnine were analyzed for roquefortine. Samples submitted to our laboratories between April and September of 1990 were evaluated.

Materials and methods

Fifty-seven diagnostic samples of stomach contents and or vomitus submitted over a six month period with a request for strychnine analysis were selected for this study. Extracts made for strychnine analysis were used for the retrospective roquefortine analysis. The extraction method used in our study did not determine the existence of other tremorgenic mycotoxins.

Roquefortine is extracted in the course of our routine alkaloid analysis which we use for strychnine analysis (7). The extract is taken up in a small volume of fresh methylene chloride and a aliquot of the extract, typically 1 μL, is injected into an HP 5840A gas chromatograph (Hewlett Packard Canada Ltd., Toronto, Ontario) coupled to an HP 5985 mass spectrometer system (Hewlett Packard) operated in the electron impact mode. If strychnine is found, the levels are determined on an HP 5830A gas chromatograph equipped with a flame ionization detector using cinchonine as an internal standard. Roquefortine will not be detected by this technique. Roquefortine is detected when 10 μL of the same solvent are evaporated in a probe which is then introduced into the mass spectrometer in the direct insertion mode. During analysis for roquefortine, the parent 389 and 320 ions resulting from loss of the isoprenyl group are continuously monitored to demonstrate the presence of roquefortine.

Results

Thirty-one samples positive for strychnine were negative for roquefortine. Six of the 26 strychnine-negative samples were positive for roquefortine.

Clinical and toxicological findings of six roquefortine-positive cases

Case 1. A nine-month-old male Shetland sheepdog was allowed to run loose in the morning. The dog had vomited prior to admission to the clinic and on presentation was undergoing seizures, panting, and paddling and exhibiting opisthotonus and nystagmus. The dog was very sensitive to noise. Treatment with intravenous barbiturates was concurrent with recovery over 48 hours. Vomitus was submitted for analysis.

Case 2. All 11-month-old female soft-coated wheaton terrier was left unsupervised in it's owner's backyard. The dog vomited prior to examination. On presentation, the dog exhibited muscle tremors, panting, hyperesthesia, and seizures. Recovery was complete by 48 hours following barbiturate treatment. Vomitus submitted for analysis contained bones which were not fed by the owner.

Case 3. A one-year-old male Labrador retriever-cross escaped from its leash in the morning. When the dog returned later that morning it was hyperactive, vomited, and began panting. A rectal temperature of $>41°C$ was recorded. The dog was very apprehensive, sensitive to noise, and hyperesthetic. Treatment with intravenous diazepam (Valium, Hoffman-La Roche Ltd., Mississauga, Ontario) did not alleviate the neurological symptoms. Following barbiturate treatment the dog was clinically normal in 24 h. Gastric content obtained by lavage was submitted for analysis.

Case 4. An 11-month-old female Dalmation was out in a pastured area with its owner who was horseback riding in the afternoon. Upon examination at the clinic that evening the dog exhibited muscle tremors, rigidity, and hyperesthesia. The dog had vomited prior to presentation to the clinic. Clinical signs did not subside with intravenous diazepam. The animal required 48 hours of treatment with barbiturates before full recovery occurred. Lavage material was submitted for analysis.

Case 5. A six-year-old female Pomeranian was off its leash. The owner noticed the dog licking at the ground in the mid-morning. By noon the dog was shaking. By 13:30 hours, severe convulsions were occurring and were observed on presentation to the clinic. No gastric lavage was attempted. Although the animal initially responded to barbiturate therapy, it was fouind dead the following morning. Postmortem examination performed by the veterinarian failed to reveal any significant findings. Stomach contents were submitted for analysis. The volume of stomach contents could not be determined from the clinic record.

Case 6. A six-year-old male silky terrier became ataxic in the evening and started to have seizures. Clinical exmination revealed panting, paddling, and opisthotonus. A gastric lavage was attempted and charcoal was given. There was no response to intravenous diazepam. Following 24 hours of barbiturate treatment the animal was euthanized. Postmortem examination revealed petechiae in the trachea and on the pleural surface of the lung. The stomach was approximately three-quarters full, containing charcoal and bones. Histological examination revealed a mild aspiration pneumonia. Additional toxicological examination for zinc phosphide, lead, metaldehyde hydrocarbons, along with brain cholinesterase levels, failed to reveal any abnormality.

Discussion

We have identified a differential diagnosis for suspected strychnine poisoning of dogs in Alberta. Although the time interval for selection of our cases

was short, we believe that it represents a realistic sample, as spring and summer temperatures would favor significant fungal growth on organic material if outside garbage is a source of poisonings. In two cases, the existence of bones in stomach contents suggested that garbage had been ingested. Previous reports have identified only moldy foods as the source of poisoning (6). Although samples were not tested from stomach contents of dead dogs that had died of other causes, the identification of roquefortine in only strychnine-negative samples suggests that this mycotoxin is not found as a background contaminant. In our opinion, the identification of roquefortine in the stomach contents of dead dogs is a significant finding.

The inability of clinicians to differentiate between strychnine and roquefortine poisoning in this study probably is due to similar clinical signs in both types of poisonings. Unawareness of roquefortine or other tremorgenic mycotoxins as a potential source of poisoning has resulted in the infrequent diagnosis of this type of toxicosis. In many cases, dogs are presented in a convulsing state and the early progression of clinical signs is probably not observed. In only one case did the clinician consider poisoning caused by agents other than strychnine. We were unable to find any reports which described the early clinical signs of roquefortine poisoning.

An interesting finding in this study was the complete absence of response to intravenous diazepam when it was given as a primary treatment in three cases. In cases of metaldehyde and strychnine poisoning, this drug is considered to be an excellent treatment (8). Although this observation needs to be verified in additional cases, this nonresponsiveness may prove to be a significant clinical observation to help veterinarians identify roquefortine and other tremorgenic mycotoxin poisoning cases.

The importance of removal of stomach contents as an initial treatment was verified by the fact that no attempt was made to remove stomach contents in the dog that died. A second dog, euthanized 24 hours after treatment had commenced, had a significant amount of stomach content present at the time of postmortem examination, despite a lavage attempt. In this study we were unable to evaluate the effect of charcoal administration. Further investigation is required to evaluate the effect of this treatment procedure in clinical cases. All four dogs that survived had vomited prior to clinical treatment. In view of these findings, an abdominal X-ray and surgical removal of stomach content should be considered as possible clinical procedures if induced vomiting is not possible.

Although we could not find specific scientific information on the metabolism of roquefortine in dogs, reports indicate that bile is the major route of excretion for penitrem A in sheep and roquefortine in rats (9,10). In view of this information, the possibility of some degree of enterohepatic recirculation and continued reabsorption may partially explain the toxicity of penitrem A and roquefortine. Failure of animals to respond to treatment within a 24 hour period may be a function of the exposure dose or a continued toxic state as a result of incomplete removal of stomach or intestinal contents. A prolonged recovery may not necessarily indicate a poor prognosis and should not be used as a clinical parameter to measure the appropriateness of euthanasia. Since fungi can produce several types of tremorgens at once, it is recommended that an examination for penitrem A and roquefortine be requested when results are negative for strychnine (11). Research in other species suggests that bile should be submitted for laboratory examination especially when stomach contents or intestinal contents are not available. If surgical removal of stomach contents is undertaken, it may also be wise to remove all available bile from the biliary tract. This could be done by expressing the gall bladder and removing the contents from the upper intestine.

Current literature did not reveal information on hematological or biochemical findings in cases of roquefortine poisoning. Significant biochemical changes associated with poisoning by penitrem A occur in dogs (12) and calves (13). Further studies evaluating the metabolism and mechanism of action of roquefortine in dogs are required. We suspect that submission of the first stool sample passed following clinical recovery may be helpful in establishing a diagnosis. Further studies are required to verify this suspicion.

A number of differential diagnoses should be considered when presented with dogs exhibiting signs of seizures, tremors, and collapse. These include poisoning by strychnine, metaldehyde, bromethalin-rodenticides, organophosphates, carbamates, chlorinated hydrocarbons, zinc phosphide, methylated xanthines (such as caffeine, theophylline, and theobromine), pyrethroids, and the insecticide diethyltoluamide. Several references covering the mechanism of action, clinical signs, and treatments used in such poisonings are available (8,14–17).

In summary, it is our opinion that roquefortine is a significant finding in the stomach contents of dead dogs and that it indicates a probable cause of death in such cases. Roquefortine alone or in combination with other tremorgens can account for clinical findings which are seen in cases of suspected strychnine poisoning. Specimens required for a laboratory diagnosis are stomach or intestinal contents. Bile should be submitted if the former are not available. In reporting these findings, we hope that other diagnostic laboratories will be encouraged to examine strychnine-negative samples for tremorgenic mycotoxins, when histories and clinical findings are suggestive of strychnine poisoning. CVJ

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